

REMARKS

The present amendment incorporates the sequence identifiers in the specification.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Appendix. Version with Markings to Show Changes Made."

Enclosed is the paper copy of the sequence listing, the sequence computer diskette, and a statement as required by 37 C.F.R. 1.821(e), (f), (g), 1.825(b) or 1.825(d).

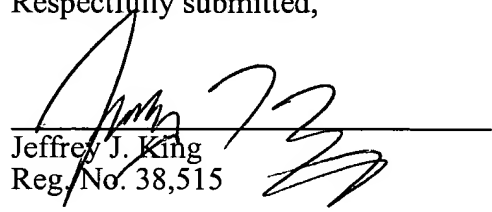
CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

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APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph beginning at page 21, line 8, has been amended as follows:

Figure 1 illustrates cloning of the N coding region of bovine PIV strains Ka or SF into HPIV3. In Fig. 1A-C, the BPIV3 N open reading frame (ORF) replaces its corresponding HPIV3 sequence in the full-length rJS antigenomic cDNA (Durbin et al., 1997a, *supra*). BPIV3 Ka and SF N genes were first amplified by RT-PCR using standard molecular biological techniques from virion RNA and subcloned as 1.9 kb fragments into pBluescript to give pBS-KaN or pBS-SFN, respectively. The HPIV3 rJS N gene was subcloned as a 1.9 kb MluI/EcoRI fragment into pUC 119 from a plasmid containing the 5' half of the rJS HPIV3 antigenome (Durbin et al., 1997a, *supra*; U.S. Patent Application Serial No. 09/083,793, filed May 22, 1998; U.S. Provisional Application No. 60/047,575, filed May 23, 1997 (corresponding to International Publication No. WO 98/53078), and U.S. Provisional Application No. 60/059,385, filed September 19, 1997, each incorporated herein by reference) to give pUC119JSN. Each N gene was modified by site-directed mutagenesis to place an NcoI and AflII site at the translational start and stop sites, respectively. The Ka and SF N genes are identical in the translational start and stop site regions and, therefore, identical mutagenesis reactions were performed on both BPIV3 N genes as depicted in 1A. Fig. 1B--Following AflII/NcoI digestion, a 1.5 kb fragment from pBS-KaN or pBS-SFN representing the BPIV3 N coding region was introduced into the NcoI/AflII window of the HPIV3 N subclone pUC119JSN-NcoI/AflII as a replacement for its HPIV3 counterpart. Fig. 1C--Each chimeric subclone was then subjected to site-directed mutagenesis to restore the sequence present in HPIV3 rJS before the translation start codon or after the stop codon and BPIV3 coding sequence immediately after the start codon and before the stop codon. This yielded pUC119B/HKaN and pUC119B/HSFN, which were used to import the BPIV3 N gene into the HPIV3 cDNA clone as shown in Fig. 2. Figure 1, Panel A, CAAAAATGTTG (SEQ ID NO. 10); GCAACTAATCGA (SEQ ID NO. 11); TAACCATGGTGA (SEQ ID NO. 12);

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GCACTTAAGCAC (SEQ ID NO. 13). Figure 1, Panel C, TAACCATGGTGA (SEQ ID NO. 12); GCACTTAAGCAC (SEQ ID NO. 13); CAAAAATGTTGA (SEQ ID NO. 14); GCAACTAGTCGA (SEQ ID NO. 15).

Paragraph beginning at page 22, line 15, has been amended as follows:

Figure 3 provides nucleotide sequences of HPIV3, BPIV3 and chimeric viruses of the invention around N translation start (A) and stop (B) codons. The position of the individual ORFs is described in the respective Genbank reports (#AF178654 for BPIV3 Ka, #AF178655 for BPIV3 SF and #Z11515) and included herein by reference. The sequences (positive-sense) flanking the translational start (A) and stop (B) codons (each underlined) in the N gene are shown for the parental recombinant HPIV3 JS (rJS), the parental biologically-derived BPIV3 Ka and SF viruses (Ka and SF), and the chimeric cKa and cSF viruses. Host-specific residues in the cKa and cSF virus sequences and their counterparts in rJS (before the start codon and after the stop codon) and SF or Ka (start codon through stop codon, inclusive) are in boldface type. Plaque-purified chimeric virus was amplified by RT-PCR from virion RNA and sequenced using the Taq Dye Deoxy Terminator Cycle kit (ABI, Foster City, CA). This confirmed that the predicted sequences were present in each chimeric virus. Figure 3A. rJS, GGA**ACTCTATAATTTC**AAAAATGTTGAGCCTATTTGATAC (SEQ ID NO. 16). Figure 3A. cKa and cSF, GGA**ACTCTATAATTTC**AAAAATGTTGAGTCTATTCGACAC (SEQ ID NO. 17). Figure 3A. Ka and SF, GAAATCCTAAGACTGTAATCATGTTGAGTCTATTCGACAC (SEQ ID NO. 18). Figure 3B. rJS, TTAACGCATTTGGAAGCAACTAATCGAATCAACATTTTAA (SEQ ID NO. 19). Figure 3B. cKa and cSF, TCAGTGCATTTCGGAAGCAACTAGTCGAATCAACATTTTAA (SEQ ID NO. 20). Figure 3B. Ka and SF, TCAGTGCATTTCGGAAGCAACTAGTCACAAAGAGATGACCA (SEQ ID NO. 21).

Paragraph beginning at page 23, line 21, has been amended as follows:

Figures 6A-6G set forth the complete positive sense nucleotide sequence (SEQ ID NO. 22) of the bovine PIV3 Ka strain.

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Paragraph beginning at page 23, line 23, has been amended as follows:

Figures 7A-7G set forth the complete positive sense nucleotide sequence (SEQ ID NO. 23) of the bovine PIV3 SF strain.

Paragraph beginning at page 24, line 5, has been amended as follows:

Figure 8C illustrates features of parental and chimeric bovine-human PIV genomes. The genomes of the chimeric rHPIV3 F_BHN_B and rBPIV3 F_HHN_H viruses and those of their parent viruses rHPIV3 JS and BPIV3 Ka are shown schematically (not to scale). Two unique restriction enzyme recognition sites, SgrAI and BsiWI, were introduced near the M and HN gene ends, respectively. The recombinant HPIV3 and BPIV3 viruses bearing these introduced restriction sites were designated rHPIV3s and rBPIV3s as indicated in Figure 8C2. Glycoprotein genes were exchanged between rHPIV3 JS and rBPIV3 Ka. The nucleotide sequence that was mutagenized is shown below each cDNA construct, with the position of the first nucleotide of each sequence indicated. The introduced *Sgr*AI and *Bsi*WI restriction sites are underlined and nucleotides that differ between HPIV3 and BPIV3 and thus identify the origin of the gene inserts are depicted in bold print. Figure 8C, Panel 1, rHPIV 3 JS, TCCACCGGTGCA (SEQ ID NO. 4), TAGACAAAAGGG (SEQ ID NO. 24). Figure 8C, Panel 1, rBPIV3 Ka, TCCAACATTGCA (SEQ ID NO. 2); AAGATATAAAGA (SEQ ID NO. 25). Figure 8C, Panel 2 rHPIV3s, CGCACCGGTGTA (SEQ ID NO. 5); TAGACGTACGGG (SEQ ID NO. 26). Figure 8C, Panel 2, rBPIV3s, TCCACCGGTGCA (SEQ ID NO. 3); AAGACGTACGGA (SEQ ID NO. 27). Figure 8C, Panel 3, rHPIV3 F_BHN_B, CGCACCGGTGCA (SEQ ID NO. 28); AAGACGTACGGG (SEQ ID NO. 29). Figure 8C, Panel 3, rBPIV3 F_HHN_H, AAGACGTACGGG (SEQ ID NO. 30); TAGACGTACGGA (SEQ ID NO. 31).

Paragraph beginning at page 69, line 1, has been amended as follows:

The complete consensus nucleotide sequence for each of the Ka or SF BPIV3 strains was determined from RT-PCR products generated from virion RNA. These sequences are set forth in Figures 6A-6G, and Figures 7A-7G, respectively. The full length cDNA encoding a complete 15456 nucleotide (nt) antigenomic RNA of BPIV3 Ka is set forth in Figures 6A-6G herein (see also GenBank accession #AF178654). The GenBank sequence for

BPIV3 kansas strain differs from the sequence of the exemplary cDNA in two positions at nucleotide 21 and 23. Both, the published sequence and the sequence in the exemplary cDNA occur naturally in kansas strain virus population with similar frequencies. The ~~former~~ cDNA used in the present example contains a sequence beginning at nucleotide 18, ACTGGTT (SEQ ID NO. 1), whereas the corresponding published sequence (GenBank accession #AF178654; Figures 6A-6G, SEQ ID NO. 22) reads ACTTGCT (~~SEQ ID NO. 1~~) (differing nucleotides at positions 21 and 23 are underscored).